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1: J Pharm Pharmacol. 1988 Sep;40(9):627-33.

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### Properties of a semicarbazide-sensitive amine oxidase in human umbilical artery.

**Precious E, Lyles GA.**

Department of Pharmacology and Clinical Pharmacology, University of Dundee, Ninewells Hospital, UK.

The metabolism of some aromatic amines by amine oxidase activities in human umbilical artery homogenates has been studied. The inhibitory effects of clorgyline showed that 5-hydroxytryptamine (5-HT) and tryptamine, 1 mM, were predominantly substrates for monoamine oxidase (MAO) type A, whereas MAO-A and B were both involved in the metabolism of beta-phenylethylamine (PEA), 100 microM, and tyramine, 1 mM. About 20-30% of tyramine and PEA metabolism was resistant to 1 mM clorgyline, but sensitive to inhibition by semicarbazide, 1 mM, indicating the presence of a semicarbazide-sensitive amine oxidase (SSAO).

Benzylamine, 1 mM, appeared to be metabolized exclusively by SSAO with a Km (161 microM) at pH 7.8 similar to that found for SSAO in other human tissues. Tyramine and PEA were relatively poor substrates for SSAO, with very high apparent Km values of 17.6 and 13.3 mM, respectively, when determined in the presence of clorgyline, 10(-3) M, added to inhibit any metabolism of those amines by MAO activities. However, kinetic studies with benzylamine indicated that clorgyline, 10(-3) M, also appears to inhibit SSAO competitively such that the true Km values for tyramine and PEA may be about 60% of those apparent values given above. No evidence for the metabolism of 5-HT or tryptamine by SSAO was obtained. The aliphatic amine methylamine was recently shown to be a specific substrate for SSAO in umbilical artery homogenates. We have used benzylamine and methylamine as SSAO substrates in histochemical studies to localize SSAO in tissue sections. (ABSTRACT TRUNCATED AT 250 WORDS)

PMID: 2907029 [PubMed - indexed for MEDLINE]

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1: J Auton Pharmacol. 1991 Oct;11(5):323-35.

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### Effect of benzylamine and its metabolites on the responses of the isolated perfused mesenteric arterial bed of the rat.

**Elliott J, Callingham BA.**

Department of Pharmacology, University of Cambridge, UK.

1. Semicarbazide-sensitive amine oxidase (SSAO) is an enzyme activity which can be found in the plasma membrane of rat vascular smooth muscle cells. We have investigated the possibility that the products of deamination by this enzyme, namely ammonia, hydrogen peroxide and the aldehyde, may be important in the modulation of the responses of vascular smooth muscle to extracellular stimuli. 2. The isolated perfused mesenteric arterial bed of the rat was used and dose-pressure response curves (DRC) to bolus injections of adrenaline (Ad) or ATP were plotted by non-linear curve fitting. The relaxant effects of carbachol (CCh), which releases endothelium dependent relaxing factor (EDRF), were studied by co-administering CCh with Ad. The effects of including the preferred SSAO substrate, benzylamine (BZ; 25 microM), in the perfusion fluid throughout the experiment and of inhibition of SSAO by treatment of rats with (E)-2-(3',4'-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145; 1 mg kg<sup>-1</sup>) 1 h before dissection, have been studied. 3. Neither BZ nor SSAO inhibition affected the DRC to ATP. BZ shifted Ad responses to the left, inhibition of SSAO increased this shift indicating that the amine, but not its metabolites, were responsible for the potentiation of the responses to Ad. DRC to CCh showed a shift to the left and a significant decrease in the Hill slope with BZ, indicative of a potentiation of low doses of CCh more than high doses. Inhibition of SSAO prevented this change and so the metabolites of BZ deamination appeared to be involved in the potentiation. 4. Ammonia generated by SSAO may contribute to the production of EDRF or hydrogen peroxide may sensitize guanylate cyclase to stimulation by EDRF and so explain these findings.

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## Semicarbazide-Sensitive Amine Oxidase (SSAO) of the rat aorta

### Interactions with some naturally occurring amines and their structural analogues

Jonathan Elliott, Brian A. Callingham and Dennis F. Sharman

Department of Pharmacology, University of Cambridge, Hills Road, Cambridge, CB2 2QD, U.K.

Received 30 August 1988; accepted 21 November 1988. Available online 15 November 2002.

**Abstract**

The influence of a number of naturally occurring amines and their structural analogues has been examined on the metabolism of radiolabelled benzylamine (BZ) by the membrane bound semicarbazide-sensitive amine oxidase (SSAO) of the rat aorta. Only primary monoamines were effective in reducing the deamination of BZ. In the phenylethylamine series, addition of hydroxyl groups to the benzene ring decreased their potency as inhibitors while addition of a hydroxyl group at the  $\beta$  position increased the inhibitory potency. Stereoselectivity of action was shown with octopamine, the L-isomer being the more active form. Kinetic analysis of these interactions showed predominantly competitive inhibition and kynuramine had the lowest  $K_i$  of 5.4  $\mu$ M. The aliphatic monoamines, isoamylamine and isobutylamine both competed with BZ. 5-Hydroxytryptamine (5-HT) was the only amine that inhibited non-competitively. Direct evidence for metabolism by SSAO of some of the competing amines such as isoamylamine, phenylethylamine, tyramine and tryptamine was obtained by fluorimetric or radio-chemical assays. The inhibitors clorgyline and (E)-2-(3',4'-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145) were used to characterise the amine oxidase activity responsible for the deamination. Octopamine and phenylethanolamine (PeOH) were not SSAO substrates and inhibited BZ metabolism in the fluorimetric assay. It is possible that the activity of SSAO is controlled by octopamine released

from sympathetic nerve endings or 5-HT released from platelets.

**Biochemical Pharmacology**

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### Semicarbazide-sensitive amine oxidase (SSAO) of the rat aorta. Interactions with some naturally occurring amines and their structural analogues.

**Elliott J, Callingham BA, Sharman DF.**

Department of Pharmacology, University of Cambridge, U.K.

The influence of a number of naturally occurring amines and their structural analogues has been examined on the metabolism of radiolabelled benzylamine (BZ) by the membrane bound semicarbazide-sensitive amine oxidase (SSAO) of the rat aorta. Only primary monoamines were effective in reducing the deamination of BZ. In the phenylethylamine series, addition of hydroxyl groups to the benzene ring decreased their potency as inhibitors while addition of a hydroxyl group at the beta position increased the inhibitory potency. Stereoselectivity of action was shown with octopamine, the L-isomer being the more active form. Kinetic analysis of these interactions showed predominantly competitive inhibition and kynuramine had the lowest  $K_i$  of 5.4  $\mu\text{M}$ . The aliphatic monoamines, isoamylamine and isobutylamine both competed with BZ. 5-Hydroxytryptamine (5-HT) was the only amine that inhibited non-competitively. Direct evidence for metabolism by SSAO of some of the competing amines such as isoamylamine, phenylethylamine, tyramine and tryptamine was obtained by fluorimetric or radiochemical assays. The inhibitors clorgyline and (E)-2-(3',4'-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145) were used to characterise the amine oxidase activity responsible for the deamination. Octopamine and phenylethanolamine (PeOH) were not SSAO substrates and inhibited BZ metabolism in the fluorimetric assay. It is possible that the activity of SSAO is controlled by octopamine released from sympathetic nerve endings or 5-HT released from platelets.

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1: J Pharm Pharmacol. 1989 Feb;41(2):97-100.

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**The enhanced daily excretion of urinary methylamine in rats treated with semicarbazide or hydralazine may be related to the inhibition of semicarbazide-sensitive amine oxidase activities.**

**Lyles GA, McDougall SA.**

Department of Pharmacology and Clinical Pharmacology, University of Dundee, Ninewells Hospital and Medical School, Scotland, UK.

The effects of amine oxidase inhibitors upon the daily urinary excretion of monomethylamine (MMA), dimethylamine (DMA), trimethylamine (TMA) and ammonia in the rat have been examined. Administration of hydralazine (5 mg kg<sup>-1</sup>) or semicarbazide (100 mg kg<sup>-1</sup>), drugs which irreversibly inhibit semicarbazide-sensitive amine oxidases (SSAO) but not monoamine oxidase (MAO), enhanced MMA excretion by around three- to six-fold above pretreatment levels, whereas no effect of pargyline (25 mg kg<sup>-1</sup>), a selective irreversible inhibitor of MAO was found. No apparent changes in DMA or TMA excretion in response to drug-treatment were observed. Ammonia excretion also was generally unchanged except for an apparent marked increase (approximately four-fold) over the 24 h following semicarbazide, a result which might be explained if ammonia is a degradation product of semicarbazide metabolism in the rat. With recent evidence that MMA is a substrate in-vitro for SSAO activities, results here may indicate that SSAO or related enzymes are involved in endogenous MMA turnover.

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1: J Pharm Pharmacol. 1985 Sep;37(9):637-43.

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### Vascular smooth muscle cells: a major source of the semicarbazide-sensitive amine oxidase of the rat aorta.

**Lyles GA, Singh I.**

Several methods have been used to study the distribution of the semicarbazide-sensitive amine oxidase (SSAO) within the wall of the rat aorta. After separation of the smooth muscle-containing layers of the tunica media from the connective tissue of the tunica adventitia, much higher specific enzyme activity (measured with 1 microM benzylamine) was found in homogenates of the media than of adventitia. Similar results were obtained for MAO-A (with 1 mM 5-HT as substrate). SSAO activity was also considerably higher in homogenates of cells (predominantly smooth muscle) isolated from medial tissue by enzymatic dissociation with collagenase and elastase compared with homogenates of cells (mostly of connective tissue origin) from the adventitia. Histochemical staining resulting from SSAO activity (with benzylamine as substrate) occurred predominantly and intensely over the tunica media in rat aortic sections, although some occasional staining of adventitial sites was also observed. Staining was prevented by the SSAO inhibitors hydroxylamine (1 microM) and semicarbazide (1 mM), but not by the MAO inhibitor, clorgyline (1 mM). These results indicate that SSAO is associated predominantly, although not exclusively, with the smooth muscle cells in the rat aorta. Our findings that beta-aminopropionitrile (BAPN) is a reversible, competitive inhibitor ( $K_i$  around  $2 \times 10^{-4}$  M) of SSAO, in contrast to the irreversible inhibition of the connective tissue lysyl oxidase by BAPN reported by others, provides further evidence that these enzymes are not identical.

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1: Prog Brain Res. 1995;106:293-303.

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### Substrate-specificity of mammalian tissue-bound semicarbazide-sensitive amine oxidase.

**Lyles GA.**

Department of Pharmacology and Clinical Pharmacology, University of Dundee, Ninewells Hospital and Medical School, UK.

Although the existence of a membrane-bound (probably plasmalemmal) semicarbazide-sensitive amine oxidase (SSAO) is well established in various mammalian tissues, and especially within vascular smooth muscle, its importance and the possible consequences of its metabolism of certain physiological and xenobiotic amines in vivo are under continuing investigation. In this respect, there are major species-related differences in substrate specificity determined in vitro, not only towards the synthetic amine benzylamine, but also towards some other aromatic amines (e.g. tyramine, tryptamine, 2-phenylethylamine, dopamine, histamine) which are possible endogenous substrates. Inhibition of SSAO can potentiate the pharmacological activity of some amines in isolated tissue (e.g. blood vessel) preparations from some species. Recent evidence has accumulated that SSAO may also be involved in metabolizing endogenous aliphatic amines such as methylamine and aminoacetone, focussing attention on the fact that the aldehyde products (formaldehyde and methylglyoxal, respectively) are potentially cytotoxic agents. Indeed, SSAO has been implicated in experimental models of cardiovascular toxicity involving conversion of the industrial aliphatic amine allylamine to acrolein. In summary, metabolism by SSAO may reduce the physiological/pharmacological effects of some amines, but the resulting metabolites (aldehydes, H<sub>2</sub>O<sub>2</sub>) may also have important actions.

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### Inhibitory actions of hydralazine upon monoamine oxidizing enzymes in the rat.

**Lyles GA, Garcia-Rodriguez J, Callingham BA.**

The inhibition by hydralazine of the clorgyline-resistant amine oxidase (CRAO) and monoamine oxidase (MAO) activities in various rat tissues has been studied. Hydralazine was a potent, time-dependent inhibitor of rat heart CRAO activity in vitro. The inhibition was not reversed by dialysis for 18 hr at 4 degrees, and only partially reversed by dialysis at 37 degrees. Dialysis at 4 degrees in the presence of pyridoxal phosphate (10(-4) M) also did not reverse the inhibition. Ex vivo inhibition of CRAO was found in heart and aorta homogenates in a dose-dependent manner after administration of hydralazine (1-40 mg/kg i.p.) to rats. In contrast, MAO-A activity was unaffected or, in some cases, significantly increased in these tissue homogenates from drug-treated animals. However, in vitro inhibition by hydralazine of both MAO-A and B activities of rat liver mitochondrial fractions was found, and these effects were fully reversible by dialysis for 18 hr at 4 degrees. Inhibition of MAO-A was competitive ( $K_i$  of  $2.5 \times 10^{-6}$  M), while inhibition of MAO-B showed complex mixed non-competitive kinetics. These results indicate that hydralazine possesses different inhibitory properties towards the various amine oxidases in rat tissues, and these actions are discussed in relation to the clinical use of the drug as an anti-hypertensive agent.

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